

IN THE SPECIFICATION

- (1) On page 7, delete paragraph 1:

~~Figures 8A and 8B illustrate fluorescence images of oligonucleotide arrays monitoring 1,650 genes in parallel (1 of a set of 4 arrays covering 6,600 genes). In Fig. 8A representative hybridization patterns of fluorescently labeled cRNA from normal (HT 125) and malignant breast (BT 474) cells are shown. The images were obtained after hybridization of arrays with fragmented, biotin labeled cRNA and subsequent staining with a phycoerythrin streptavidin conjugate. Bright rows indicate messages present at high levels. Low level messages (1-10 copies/cell) are unambiguously detected based on quantitative analysis of PM/MM intensity patterns. In the lower portion of the Figure a magnified view of a portion of the array highlighting examples of altered gene expression between BT 474 and HT 125 is shown. In area 1, induced (>10 fold change in hybridization intensity) genes are shown, in area 2, unchanged (<2 fold change in hybridization intensity) are shown, and in area 3 repressed (>10 fold change in hybridization intensity) are shown. Fig. 8B illustrates zoom in images of genes 1, 2, & 3 in (A) as 20 probe pairs of perfect matched (PM) and single base mis-matched (MM) oligonucleotide probe cells. Average fluorescence intensity difference for PM-MM in HT 125 versus BT 474 (normalized to β Actin and GAPDH signals) are shown in the three rows. In row 1 (Her2/neu oncogene) the average intensities are 111 versus 5,127; in row 2 (laminin receptor) 3,495 versus 6,088; and in row 3 (galectin 1) 7,952 versus undetected.~~

- (2) On page 7, delete paragraph 2 and replace it with the following paragraph:

Figure [[9]] 8A and 8B illustrates expression profiles of subset of genes from normal versus malignant breast cells. Average perfect match-mismatch (PM-MM)intensity differences (normalized to β -Actin and GAPDH signals) were plotted for the genes ~~highlighted in Figure 8A~~ that demonstrated greater than a 2-fold difference in hybridization signals between HT-125 and BT-474. Values for signals off scale are indicated.

- (3) On page 7, delete paragraph 3, which spans page 8, and replace it with the following paragraph:

~~Figure 10 illustrates p53 sequence analysis and mutation detection by hybridization. In Figure 11A, an image of the p53 genotyping array hybridized to 1,490 bp of the BT 474 breast~~

carcinoma p53 gene (left) is shown. A zoom in view of hybridization patterns of p53 wild-type reference and BT-474 DNA in a region of a G→A single base mutation in BT-474 is shown at the right. In each column are 4 identical probes with an A, C, G or T substituted at a central position. The hybridized target sequence identified based on mismatch detection from left to right as the complement of the substitution base with the brightest signal. The G→A transition seen in BT-474 is accompanied by a loss of signal at flanking positions as these probes now have a single base mismatch to the target distinct from the query position. Fig. 10B (top) 9A, comparison of wild-type reference (black) and BT-474 p53 gene (red) hybridization intensity patterns from sense (above) and anti-sense strands (below) in the region containing a mutation. The area shown demonstrates the “footprint” and detection of a single-base difference between the samples (vertical green paragraph). GeneChip data analysis output is shown (bottom) (Fig. 9B) that unambiguously identifies a G→A base change at nucleotide 1,279 of p53 in BT-474 resulting in a glutamic acid to lysine amino acid change in exon 8 (DNA binding domain). The upper portion of output displays the p53 wild-type reference sequence. Aligned outputs of wild-type p53 control and BT-474 samples are shown.

- (4) On page 8, insert the following paragraph before paragraph 1:

Fig. 10A –10M list genes transcriptionally induced and transcriptionally repressed in BT-474 vs. HT-125 cell lines.

- (5) On page 52, delete the first full paragraph and replace it with the following paragraph:

Figure 8A (top panel) shows representative hybridization Hybridization patterns of total message from normal and malignant breast cells to sets of 20 probe pairs from 1,650 gene sequences (one array of a set of 4 encompassing 6,600 human genes) were obtained. Clear examples of unchanged and altered patterns of gene expression can be observed by visual comparison of the fluorescence intensities of probe sets from these two samples. The quantitative analysis of hybridization patterns is based on the assumption that for a specific mRNA the perfect-matched (PM) probes will hybridize more strongly on average than their mismatched (MM) partners (Fig. 8B). The average difference in intensity between PM and MM hybridization signals is computed together with the average of the logarithm of the PM/MM

ratios for each probe set. These values are then used to determine the relative copy number of a detected message.

(6) On page 52, delete paragraph 2 and replace it with the following paragraph:

Added biotinylated control cRNAs (*E. coli* biotin synthetase genes *bioB*, *bioC*, *bioD* and bacteriophage P1 *Cre* recombinase) at known concentrations served as internal standards to allow relative quantitation of fluorescence intensities for estimates of copy number per cell. Spiking experiments were performed to investigate the absolute hybridization intensity range between multiple RNAs at known concentrations. When 32 individual cRNAs were spiked at levels ranging from copy numbers of 1:100,000 to 1:30,000 in the background of total cellular mRNA, absolute hybridization intensities were within a 2-fold range for all targets tested. Added biotinylated control oligonucleotide together with endogenous cellular RNA messages (e.g. β -Actin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH)), allowed for normalization of experimental variation and array hybridization. Intensities from the spiked standards in the presence of total cellular target demonstrated sensitivities as high as 1:100,000, corresponding to a few copies per cell. Comparison of hybridization signal intensities, that ranged over 4-orders of magnitude, revealed all categories of message expression changes including repressed (>10-fold down), down-regulated (<10-fold down), up-regulated (<10-fold up) and induced (>10-fold up) mRNAs between normal and malignant cells as shown in Fig. [[9]] 8A and 8B.

(7) On page 53, delete the first full paragraph and replace it with the following paragraph:

Genes that are repressed and induced/activated may provide a particularly good starting point to decipher the molecular pathways involved in programs of tumorigenesis. To identify the genes falling into each of these two categories we sorted the normal and malignant message populations to identify those genes that demonstrated a 10-fold or greater difference in message intensities. This analysis revealed 168 genes repressed and 137 genes activated in BT-474 when compared to HT-125, as shown in Table 3 (Figure ~~9A and 9B~~ 10A –10M). 260 of the messages displaying differential expression corresponded to GenBank human full length genes, 45 to ESTs with homologies to other eukaryotic or viral genes.